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Cadmium Ecophysiology in Seven Stonefly (Plecoptera) Species: Delineating Sources and Estimating Susceptibility

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A major challenge in ecotoxicology lies in generating data under experimental conditions that are relevant to understanding contaminant effects in nature. Biodynamic modeling combines species-specific physiological traits to make predictions of metal bioaccumulation that fare well when tested in the field. We generated biodynamic models for seven predatory stonefly (Plecoptera) species representing the families Perlidae (5) and Perlodidae (2). Each taxon was exposed to cadmium independently via diet and via solution. Species varied approximately 2.6 fold in predicted steady-state cadmium concentrations. Diet was the predominant source of accumulated cadmium in five of the seven species and averaged 53.2 ± 9.6% and 90.2 ± 3.7% of net Cd accumulation in perlids and perlodids, respectively. Differences in Cd bioaccumulation between the two families were largely driven by differences in dissolved accumulation rates, which were considerably slower in perlodids than in perlids. We further examined the subcellular compartmentalization of Cd accumulated from independent aqueous and dietary exposures. Predicted steady-state concentrations were modified to only consider Cd accumulated in metal-sensitive subcellular compartments. These values ranged 5.3 fold. We discuss this variability within a phylogenetic context and its implications for bioassessment.

Introduction

Human activities have resulted in trace metal contamination of aquatic ecosystems worldwide (*1*). As a result, benthic community structure, more specifically, macroinvertebrate density and species diversity, can be adversely impacted (*2*, *3*). Aquatic insects constitute a large majority of the invertebrate species pool in most lotic systems and play fundamental roles in stream ecosystem function (*4*). Accordingly, aquatic insects are widely used in bioassessment (*5*, *6*), ecological risk assessments (*7*), and as biomonitors of contaminant exposure (*8*, *9*). Aquatic insects have proven to be invaluable in evaluating the integrity of ecosystems impacted by anthropogenic stressors such as trace metals, yet the relationship between insect physiology and contaminant bioaccumulation has received surprisingly little attention.

Biodynamic modeling has emerged as a powerful tool for predicting trace metal bioaccumulation differences among species (*10*). The dynamic multipathway bioaccumulation model (DYM-BAM) integrates laboratory derived rate constants of accumulation and elimination. These trace metal accumulation and elimination processes can be considered species-specific physiological traits. The predictive power of biodynamic modeling stems from the use of environmentally relevant exposure conditions and the incorporation of metal accumulation from both aqueous and dietary sources. Bioaccumulation studies that neglect dietary exposures may not provide ecologically relevant forecasts of metal bioaccumulation. Numerous studies have acknowledged diet as a major exposure route that significantly contributes to metal bioaccumulation in invertebrates such as bivalves (*11*, *12*), freshwater crustaceans (*13*, *14*), and insects (*15*, *16*).

Two paradigms currently exist for understanding metal toxicity to aquatic organisms: the biotic ligand model (BLM) and biodynamic modeling. The BLM is emerging as a powerful tool for understanding site-specific acute metal toxicity and has been most effective when applied to taxa that experience toxicity as a result of metal binding to biotic ligands on the gill surface (*17*). The BLM approach appears best applied to species with relatively low acute to chronic ratios. Aquatic insects, however, do not appear to be acutely sensitive to trace metals relative to many other faunal groups (*18*). Yet many insects seem to be affected by chronic metal exposures in nature (*19*) where their diets may be a significant source of accumulated metal. Biodynamic modeling is well suited for understanding metal bioaccumulation differences among insect species because it considers all exposure routes. However, the physiological consequences of accumulated metal remain poorly understood in this group. An underlying premise in biodynamic modeling is that metal toxicity occurs when metal influx exceeds the organism's ability to effectively eliminate or detoxify excess metal (*2*, *20*). Therefore, when associated with other approaches, biodynamic modeling can be augmented to better predict metal toxicity (*19*). One such approach is to examine the subcellular compartmentalization of accumulated metals (*19*, *21*−*23*). The subcellular partitioning of metals within an organism is an additional physiological trait that when used in conjunction with biodynamic modeling can help explain differences in trace metal susceptibility among taxa (*19*, *21*) and is a first step in attempting to link metal bioaccumulation to toxicity.

This study is the first to apply biodynamic modeling to stoneflies, a diverse and ecologically important group in lotic ecosystems. We generated biodynamic models of Cd bioaccumulation for seven species of predatory stoneflies (Plecoptera) representing the families Perlidae and Perlodidae. Models allowed us to examine bioaccumulation differences among taxa and assess the relative importance of aqueous and dietary cadmium exposures. Furthermore, we evaluated the subcellular compartmentalization of Cd independently from each exposure route. Thus, we were able to predict differences in net Cd bioaccumulation among species, and also quantities of Cd associated with subcellular biomolecules that are potentially susceptible to metal insult (hereafter referred to as metal-sensitive compartments). This allowed us to estimate Cd susceptibility differences among taxa. The distribution of these physiological traits in the context of a phylogenetic framework is discussed.

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TABLE 1. Taxa, Source Location, Mass and Numbers of Individuals Used in Bioaccumulation Experiments[a]

family	genus species	sampling location	mass (mg) ± SE	*n* aq.	*n* diet
Perlidae	*Claassinea sabulosa*	40°40′01″N, 105°13′32″W	144.3 ± 27.6	20	20
Perlidae	*Paragnetina* sp.	36°22′47″N, 81°09′11″W	144.6 ± 15.0	14	17
Perlidae	*Calineuria californica*	37°17′20″N, 122°04′20″W	105.1 ± 10.1	20	20
Perlidae	*Acroneuria abnormis*	36°22′47″N, 81°09′11″W	216.5 ± 18.5	16	20
Perlidae	*Hesperoperla pacifica*	37°17′20″N, 122°04′20″W	265.6 ± 13.3	10	14
Perlodidae	*Isogenoides hansoni*	36°22′10″N, 80°59′30″W	108.5 ± 13.2	6	16
Perlodidae	*Baumanella alameda*	37°04′35″N, 121°28′02″W	34.9 ± 1.6	9	19

[a] Masses reported here are from animals used in dietary experiments.

Materials and Methods

Sample Collection and Handling. Larvae were field collected from wadable riffles using a D-frame kick-net from nonpolluted streams in California, Montana, Colorado and North Carolina (Table 1). Specimens were sorted and placed in Whirl Pac bags containing streamwater and substrate and were transported to the lab in coolers. In the lab, larvae were placed in clean, acid-washed 300 mL Nalgene polypropylene exposure chambers containing aerated ASTM artificial soft water (48 mg L^{-1} $NaHCO_3$, 30 mg L^{-1} $CaSO_4 \cdot 2H_2O$, 30 mg L^{-1} $MgSO_4$, and 2 mg L^{-1} KCl) and were acclimated to laboratory conditions at 14 °C for a minimum of 48 h. Larvae were deprived of food during this period. Following acclimation, insects were randomly divided into three groups. Group 1 animals were used to estimate assimilation efficiency and depuration kinetics from dietary exposure (see below). Group 2 animals were used to investigate the subcellular compartmentalization of dietborne Cd (see below). Group 3 animals were used to examine dissolved Cd uptake and depuration kinetics as well as subcellular Cd compartmentalization (see below). Only apparently healthy, active, and intact larvae were used.

Assimilation Efficiency and Depuration Kinetics. To investigate dietary cadmium accumulation kinetics, approximately 10 larvae were weighed and individually placed in 50 mL Nalgene polypropylene exposure chambers containing artificial soft water and a 2 × 3 in. piece of Teflon-coated mesh as artificial substrate. Each larva was hand fed one contaminated prey item: the freshwater oligochaete, *Lumbriculus variegatus*. This species has several characteristics that make it ideally suited for use as food item ((*24*), Xie and Buchwalter, unpublished data). Worms were uniformly labeled with ^{109}Cd via aqueous exposure lasting 5 days and were assayed for radioactivity to ensure uniformity in Cd levels. Worms did not achieve steady-state cadmium concentrations during this exposure period. Exposure water was prepared by adding $^{109}CdCl_2$ (9 ng mL^{-1} in 0.1 N HNO_3) and $CdCl_2$ (1 μg mL^{-1} in 0.1 N HNO_3) to achieve a final Cd concentration of 518 ng L^{-1} (4.6 nM), with a specific activity of ~2.14 μCi L^{-1}. Sodium hydroxide was added to adjust the pH to ~6.85. Immediately following ingestion of the contaminated prey, larvae were thoroughly rinsed and assayed for initial radioactivity. Then, larvae were placed in individual exposure chambers, provided with clean prey ad libitum, and assayed for radioactivity daily over 10 days of depuration. Water was refreshed daily to minimize reuptake of effluxed metal. Assimilation efficiencies (AE) were estimated as the proportion of initial tracer remaining in the larvae after fecal excretion. Ingestion rates (IRs) were determined by averaging the daily difference between the mass of prey provided and the mass of prey remaining over 7 days. IRs were derived from 6−10 individuals per taxon. The efflux rate constants (k_e) were determined by fitting data from the depuration phase to a nonlinear least-squares regression model (*25*).

Subcellular Compartmentalization of Cd from Dietary Sources. Approximately 10 individuals (depending on availability) were fed contaminated prey ad libitum until each had accumulated ≥2500 cpm (~5 days, although feeding durations varied based on IR). Water was refreshed daily to minimize potential aqueous exposure from effluxed metal. To reduce the potentially confounding effects of radioactive gut contents, larvae were fed clean prey ad libitum for 2 days, followed by two additional days of fasting. Following this depuration period, insects were rinsed, weighed, assayed for radioactivity, and stored in liquid nitrogen. Three to four replicate homogenates were used per species to assess subcellular compartmentalization. Each homogenate consisted of one to three individuals, depending on body size and availability. The homogenates were separated into five operationally defined subcellular compartments by differential centrifugation and heat treatment according to methods described by Cain et al. (*21*) (adapted from Wallace et al. (*22*)). All fractions were assayed for radioactivity. We assumed that Cd in the heat-stable protein fraction was largely bound to metallothionein-like proteins (*26*, *27*), while the heat-denatured fraction represented a variety of larger cytosolic proteins. We refer to the heat stable and heat-denatured protein fractions as metallothionein-like protein (MTLP) and non-MTLP, respectively. Proportions of recovered radioactivity in each of the five subcellular compartments were summed into operationally defined metal-sensitive and detoxified compartments. The metal-sensitive compartment comprised the non-MTLP, microsomal and organelle fractions, each containing sites potentially susceptible to Cd binding. The detoxified metal compartment consisted of cadmium associated with MTLP. Cadmium in the cell debris fraction was interpreted as being associated with biologically inert tissue such as chitin.

Aqueous Exposures. Methods are described previously in Cain et al. (*28*) and Buchwalter et al. (*19*). Exposure water was prepared from the same cadmium stock solution used in preparation of the prey (see above). One mL aliquots of exposure water were assayed for radioactivity and measured for pH to ensure uniformity of exposure conditions. Approximately 15−20 larvae were incubated in this Cd solution which was refreshed after days one and three of exposure. Following 5 days of accumulation, half of the insects were placed in clean artificial soft water and efflux was quantified by in vivo gamma counting daily for an additional 10 days (see above). The remaining larvae were frozen for subcellular fractionation.

Radioactivity Measurement. All radioisotope activity was measured using a Wallac Wizard 1480 gamma counter. Insects were individually weighed and placed into 20 mL counting vials containing ~15 mL of 15 °C artificial soft water. A counting time of 3 min yielded <5% counting errors. Appropriate corrections were made for radioactive decay and counting efficiency.

Biodynamic Modeling. The dynamic multipathway bioaccumulation model (DYM-BAM) is discussed in detail by Schlekat et al. (*25*) and Luoma et al. (*10*). The differential equations describing the processes of metal influx and efflux from both dissolved and dietary exposures can be solved as an expression of the individual's steady-state concentration as in eq 1,

$$C_{ss} = \frac{(k_u \times C_W) + (AE \times IR \times C_F)}{k_e} \quad (1)$$

where k_u is the uptake rate constant from solution (L $g^{-1}day^{-1}$), C_W is the concentration of metal in solution (μg

TABLE 2. Biokinetic Models Parameters for Seven Stonefly Species[a]

genus species	AE (%)	IR (g g^{-1} day^{-1})	k_u (L g^{-1} day^{-1})	k_e (aqueous) (day^{-1})	k_e (dietary) (day^{-1})	[Cd]ss (μg g^{-1}) min	med	max
Claassenia sabulosa	77−93	0.03−0.09	0.100 ± 0.020	0.082 ± 0.007	0.091 ± 0.034	0.67	1.42	3.14
Paragnetina sp.	68−80	0.01−0.06	0.164 ± 0.001	0.085 ± 0.003	0.118 ± 0.027	0.98	1.28	1.90
Calineuria californica	81−89		0.102 ± 0.012	0.081 ± 0.032	0.049 ± 0.008	0.84	2.17	6.24
Acroneuria abnormis	75−89	0.03−0.06	0.211 ± 0.029	0.087 ± 0.003	0.076 ± 0.017	1.22	1.97	5.50
Hesperoperla pacifica	74−90	0.04−0.10	0.059 ± 0.012	0.082 ± 0.020	0.078 ± 0.013	0.51	1.43	4.26
Isogenoides hansoni	57−73	0.05−0.12	0.027 ± 0.003		0.110 ± 0.027	0.29	0.84	2.01
Baumanella alameda	68−79		0.009 ± 0.001		0.065 ± 0.014	0.38	1.07	2.45

[a] Taxa above the dashed line are from the perlid family, taxa below are from the perlodid family. Ranges of steady-state cadmium concentrations were modeled based on 0.52 μg L^{-1} Cd in water of and 1.53 μg Cd g^{-1} (wet weight) in food. Values for the model parameters are reported as mean ± SE. A range of ingestion rates are reported here. Mean IR for all other taxa were used in modeling bioaccumulation in *Calineuria* and *Baumenella*. On average, efflux rates were similar for both exposure routes, with greater error estimates associated with dietary exposures. However, aqueous and dietary efflux rate constants were significantly different in *Calineuria*.

L^{-1}), AE is assimilation efficiency, IR is ingestion rate (g g^{-1}day^{-1}), C_F is the concentration in the food (μg g^{-1}), and k_e is the efflux or elimination rate constant (proportional daily loss, day^{-1}). Growth dilution, k_g, was not considered in these experiments. Under the assumption that whole organism exposure is an additive function of both exposure pathways (*29*), the relative importance of exposure routes was quantified by separating their respective parameters. Equations 2 and 3 represent the expressions used to calculate the steady-state concentrations from waterborne and dietborne exposures, respectively,

$$C_{ss(water)} = \frac{(k_u \times C_w)}{k_{e\ (water)}} \quad (2)$$

$$C_{ss(food)} = \frac{(AE \times IR \times C_f)}{k_{e\ (food)}} \quad (3)$$

where $k_{e(water)}$ and $k_{e(food)}$ are efflux rate constants derived from aqueous and dietary exposures, respectively.

Statistical Analysis. SAS system for Windows (version 8.10) software was used in statistical analysis. *T* tests were performed to compare mean bioaccumulation parameters between families and between exposure routes. Experimental data were normally distributed. Homogeneity of variance was verified using Bartlett's test for equal variances prior to application of parametric statistics on individuals within a given species.

Results

Assimilation Efficiency (AE). Cadmium AE estimates for seven taxa ranged from 57 to 93% (Table 2) and were slightly higher on average in Perlidae (81.6 ± 2.0%) than in Perlodidae (69.5 ± 4.5%).

Cd Elimination Kinetics. Elimination rate constants were derived independently from dissolved and dietary experiments. Overall, efflux rate constants were very similar among species and between exposure routes. For example, the average $k_{e(food)}$ (0.084 ± 0.009 day^{-1}) was similar to $k_{e(water)}$ (0.083 ± 0.001 day^{-1}), and there were no apparent differences in $k_{e(food)}$ between families ($p = 0.823$) (Table 2). However, $k_{e(water)}$ could not be estimated for the perlodids *Baumanella alameda* and *Isogenoides hansoni* because dissolved Cd influx was so slow in these organisms that levels of Cd loading sufficient for efflux studies were not achieved after 5 days of Cd exposure (Figure 1). Within a given taxon, $k_{e(food)}$ and $k_{e(water)}$ did not differ significantly in four of five taxa (Table 2). In *Calineuria californica* $k_{e(food)}$ and $k_{e(water)}$ were significantly different ($p = 0.0326$), however, the difference was relatively small (~3% of proportional daily loss).

Dissolved Cd Uptake. In contrast to dietary Cd assimilation, there were significant differences in dissolved cadmium uptake (Figure 1) and associated rate constants (k_u) (Table 2) among species (one way ANOVA, $p < 0.0001$). Uptake rate constants ranged 23 fold among all species, with perlids (0.127 ± 0.027 L g^{-1} d^{-1}) generally having faster uptake rates than perlodids (0.018 ± 0.009 L g^{-1} d^{-1}). While this difference was marginally significant ($p = 0.06$), analyses including an additional perlid (*Doroneuria baumanni*) and an additional perlodid (*Skwala* sp.) showed significant differences in k_u values ($p = 0.04$) (these taxa are not addressed in this paper because dietary studies were not performed). Uptake rate constants were highly variable within the Perlidae, contrasting sharply with consistency in k_e observed in this family.

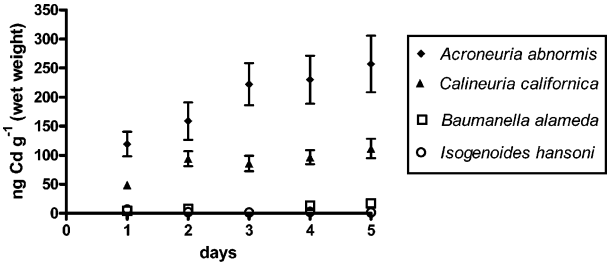

FIGURE 1. Short-term dissolved cadmium accumulation in selected taxa. Accumulation was generally faster in perlids (closed symbols) than in perlodids (open symbols). These taxa were chosen to illustrate the variation in Cd uptake rates. Organisms have not reached steady-state concentrations. Deviation from zero was significant in the slopes of all four taxa.

Biodynamic Modeling. For each taxon, predicted ranges of steady-state cadmium concentrations were modeled assuming a Cd concentration of 0.52 μg Cd L^{-1} in water and 1.53 μg Cd g^{-1} in food. Predictions were generated using the minimum, median, and maximum estimates of model parameters (Table 2). For example, the lower range of predicted Cd concentration was derived using the minimum estimated AE, IR, and k_u, and the maximum estimated k_e derived from each taxon. Median Cd concentrations ranged 2.6 fold among all species, and averaged 1.65 ± 0.18 μg g^{-1} in perlids and 0.96 ± 0.12 μg g^{-1} in perlodids. This difference is largely accounted for by differences in dissolved Cd accumulation between the families. The average dietary derived [Cd] was predicted to be similar between perlids 0.90 ± 0.20 μg g^{-1} and perlodids 0.89 ± 0.11 μg g^{-1}. In five out of the seven taxa tested here, diet was the predominant cadmium exposure route (Figure 2). On average, diet was a greater contributor to net Cd bioaccumulation in the perlodid family (90.2 ± 3.7%) than in the perlids (53.2 ± 9.6%).

Cd Compartmentalization. Cadmium compartmentalization varied within and among the two stonefly families (Table 3) and also varied by route of exposure (Figure 3). In all seven taxa, a greater proportion of Cd accumulated from the aqueous route was distributed in the cell debris com-

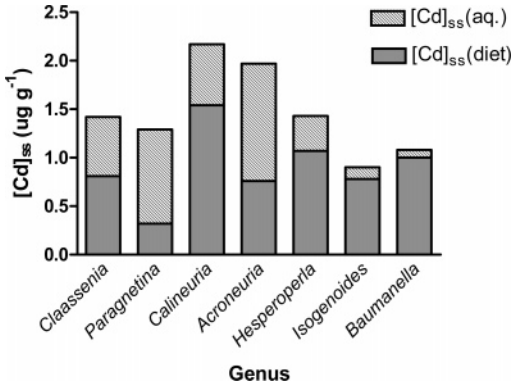

FIGURE 2. The relative importance of dietary vs aqueous exposure routes and total modeled cadmium accumulation. Diet contributed between 25−93% of net accumulation (based on median model parameters) and was the dominant exposure pathway in five out of seven taxa studied.

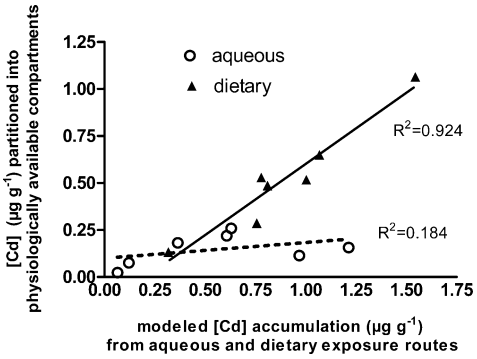

FIGURE 3. Relationship between total and physiologically available cadmium bioaccumulation. Each species is represented by two points depicting its aqueous and dietary accumulation. Diet derived Cd efficiently targets potentially metal-sensitive compartments relative to the dissolved route of exposure. These data suggest that dietborne metal is potentially more toxic than Cd accumulated from solution.

partment, likely representing integument-associated metal. Thus, the relative proportions of Cd in both MTLP and metal-sensitive compartments were uniformly higher in dietary exposures compared to dissolved exposures. However, an interesting trend emerges if the modeled steady-state Cd concentrations derived from each exposure route are plotted against the concentrations of Cd projected in metal-sensitive fractions (Figure 3). Across taxa, potentially toxic, diet derived cadmium concentrations were highly correlated with total Cd accumulation ($p = 0.001$). However, this trend was not apparent in the dissolved route of exposure ($p = 0.335$). These results suggest that even if the contributions from both exposure routes were equivalent, dietborne Cd is potentially more harmful than dissolved Cd, because more dietborne Cd is accumulated in metal-sensitive fractions.

Combining Biokinetic Modeling with Subcellular Fractionation. Modeled steady-state concentrations based on biokinetic parameters were modified to consider only cadmium in metal-sensitive subcellular compartments (Figure 4). These concentrations varied greater than 5 fold among all seven taxa, with the greatest variation observed among the Perlidae. Under these experimental conditions, cadmium accumulation in metal-sensitive compartments varied from 0.25 μg g^{-1} in *Paragnetina* sp. to 1.32 μg g^{-1} in *Calineuria californica,* suggesting that generalizations about susceptibility made at the family level are likely inappropriate for this group. The two perlodids examined were similarly predicted to accumulate 0.61 and 0.55 μg Cd g^{-1} in metal-sensitive compartments in *Isogenoides hansoni* and *Baumanella alameda* respectively.

Discussion

Biodynamic modeling has provided a framework for using short-term standardized protocols in the lab to make accurate predictions of metal bioaccumulation in nature (*10*). This approach is particularly useful for species that are not amenable to long-term handling in the laboratory (*19*). In aqueous cadmium exposures, for example, estimates of the time required for species to achieve steady-state body burdens (relative to the ambient water concentrations used in these studies) range from 51 to 110 days, whereas rate constants used to make these predictions were generated in only 15 days. Furthermore, biodynamic modeling allows

TABLE 3. Subcellular Cd Compartmentalization in Seven Plecopteran Species[a]

	fractions (%)				
	detoxified	metal-sensitive			inert
genus species	MTLP	non MTLP	organelle	microsome	cell debris
Claassenia sabulosa	8.8 ± 3.6 14.0 ± 2.0	15.5 ± 3.0 38.3 ± 3.0	14.1 ± 5.4 6.5 ± 2.6	6.7 ± 1.2 15.2 ± 1.5	52.9 ± 4.0 26.0 ± 3.8
Paragnetina sp.	31.8 ± 0.7 11.5 ± 2.3	2.6 ± 0.2 24.5 ± 3.5	6.9 ± 3.7 11.1 ± 4.0	2.4 ± 0.4 6.4 ± 0.5	56.2 ± 3.4 46.5 ± 2.3
Calineuria californica	3.4 ± 0.7 14.0 ± 2.5	21.9 ± 2.4 50.3 ± 4.0	7.9 ± 1.6 8.7 ± 0.9	11.5 ± 0.4 9.9 ± 2.0	55.4 ± 6.7 17.4 ± 1.7
Acroneuria abnormis	33.9 ± 1.2 31.6 ± 0.9	2.5 ± 0.1 21.4 ± 3.3	4.7 ± 0.6 8.2 ± 1.1	5.8 ± 0.3 8.2 ± 0.5	53.2 ± 1.7 30.6 ± 0.8
Hesperoperla pacifica	7.7 ± 4.8 22.3 ± 4.4	32.7 ± 5.6 49.6 ± 6.3	8.8 ± 0.0 3.7 ± 0.2	8.8 ± 1.5 7.6 ± 2.1	41.9 ± 3.9 16.8 ± 3.3
Isogenoides hansoni	10.3 ± 0.3 23.3 ± 2.5	48.7 ± 0.3 61.3 ± 6.1	7.2 ± 0.3 3.0 ± 1.3	7.9 ± 0.5 3.7 ± 0.9	25.9 ± 0.5 8.6 ± 1.2
Baumanella alameda	29.4 ± 3.1 36.8 ± 6.8	15.4 ± 2.8 32.2 ± 8.2	10.2 ± 0.8 8.6 ± 1.1	10.8 ± 4.3 10.8 ± 1.4	34.3 ± 3.8 16.6 ± 1.9

[a] Cadmium body burdens were subdivided into five operationally based fractions by differential centrifugation and heat treatment. Cadmium associated with organelles, microsomes, and heat labile cytosolic proteins (non-MTLP) are considered potentially toxic. The subcellular distributions of Cd after dissolved (gray) and dietary (white) exposures are reported as a percentage ± SE. Data are normalized to recovered radioactivity. Recoveries ranged from 70 to 80%, which is typical for sequential procedures involving multiple transfers. The first five taxa are from the perlid family. The final two taxa are from the perlodid family.

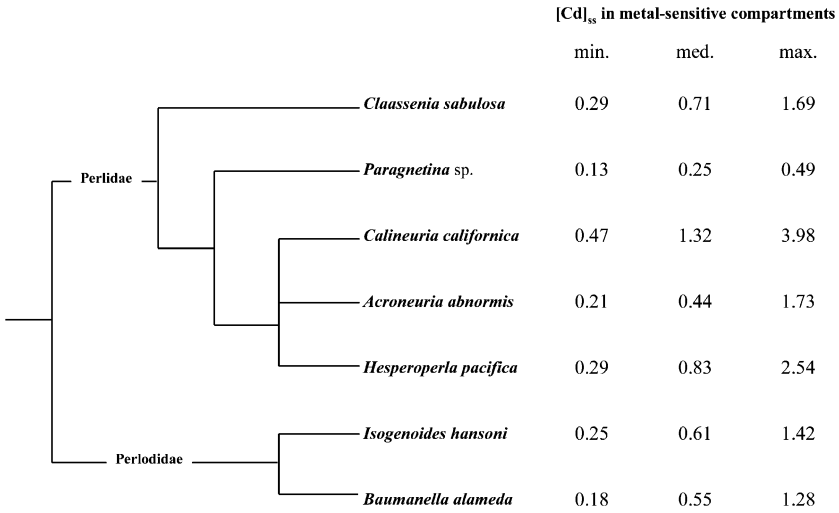

FIGURE 4. Predicted ranges of Cd steady-state concentrations (μg g^{-1}) wet weight in metal-sensitive subcellular compartments. Subcellular disposition of accumulated cadmium is used to infer susceptibility differences among taxa. The cladogram on the left indicates taxonomic relationship (source: The Tree of Life Web Project: http://tolweb.org/tree/phylogeny.html). Considerable variation among taxa suggests that family level generalizations about susceptibility are unsupported.

researchers to determine the relative importance of dissolved and dietary metal exposures and explains why species vary so greatly in their accumulation of trace metals.

The overwhelming majority of species for which biodynamic models have been generated are marine invertebrates (*29*, *30*). We chose stoneflies for these studies because they are dominant members of stream communities in many parts of the world. Perlids are widespread in the northern hemisphere and can be found in Africa and South America. Perlodids are found spanning the Holoarctic ecozone (*31*). Furthermore, stoneflies are a common focus in bioassessments of freshwater ecosystems. To our knowledge, biodynamic models incorporating the dietary route of exposure have only been generated for two lake dwelling predatory insects: the dipteran, *Chaoborus punctipennis* (*15*) and the megalopteran, *Sialis velata* (*16*).

Our k_e estimates fall within a range observed in many invertebrates. Among aquatic insects, $k_{e(water)}$ estimates range from approximately 0.14 to 0.24 day^{-1} in the caddisflies, *H. californica* (*28*), *H. bettini* (*32*), and *Rhyacophila* sp. (*19*). In a number of species of Chaoborus, $k_{e(water)}$ has been reported to range from 0.003 to 0.08 day^{-1} (*33*). It is often assumed that elimination rate constants from dietary and aqueous exposures are comparable and differences may be small enough to be considered negligible (*10*). Our results validated this assumption in predatory stoneflies. The inter-specific variation in cadmium k_u is also consistent with other studies. For example, k_us range from 0.02 to 0.55 L g^{-1}d^{-1} among several mayfly taxa and from 0.04 to 0.42 L g^{-1}d^{-1} among four caddisfly taxa (Buchwalter, unpublished data). The differences in k_u were large enough to explain much of the difference in modeled Cd accumulation among stoneflies in our studies.

Our work with stoneflies is consistent with other predatory insects, in that diet is a major source of accumulated metal (*15*, *16*, *34*). One explanation for diet being such an important contributor to Cd accumulation in these taxa is that assimilation efficiencies were quite high. This is consistent with other studies reporting AE in predatory invertebrates (*34*, *35*). We speculate that the high AEs observed in predatory insects in general may be associated with feeding strategy. Predators that consume a significant percentage of their body weight in one meal and then can fast for several days may benefit from more efficient assimilation of nutrients, proteins, lipids, and essential metals, particularly when prey availability varies spatially and temporally.

Also with respect to assimilation efficiency, it has been suggested that the subcellular distribution of metal within the prey affects trace metal bioavailability, with cytosolic metal typically being highly available for trophic transfer (*36*−*38*). Subcellular compartmentalization of the prey has indicated that after 5 days of dissolved cadmium accumulation, up to 70% of the Cd accumulated by *L. variegatus* was distributed in cytosolic compartments (Xie and Buchwalter, unpublished data). Interestingly, during depuration of dietborne Cd, many individuals exhibited slow, steady tracer elimination prior to observed fecal loss. This phenomenon suggests highly efficient transport of Cd from the midgut to the hemeoceole, with a small proportion of accumulated Cd subsequently removed by malphigian tubules during the production of urine. This process is consistent with studies in the blowfly (*39*) in which calcium uptake at the midgut was unregulated even in hypercalcemic individuals. Calcium content in the hemolymph was regulated by the malphigian tubules (eliminating excess), rather than by reducing Ca accumulation. This may help explain the high Cd assimilation rates we observed in stoneflies.

In spite of diet being the dominant exposure pathway in five out of seven taxa, these predicted dietary contributions to net Cd accumulation can be considered highly conservative estimates. Larvae were fed a simplified diet of oligochaetes exposed to Cd for only 5 days with an average Cd concentration of 1.53 μg Cd g^{-1} wet weight (cadmium steady-state concentration in these worms were projected to be almost 10-fold higher at 15.1 μg Cd g^{-1}). In nature, stonefly larvae feed on a variety of prey items including mayflies which can vary widely in their metal concentrations (*9*). Likewise, in predicting Cd steady-state concentrations, the aqueous contribution can be considered an overestimate, given that the dissolved uptake experiments were performed in the absence of dissolved organic carbon, favoring free Cd ion concentrations.

Despite its utility in accurately predicting bioaccumulation differences among species, biodynamic modeling does not directly establish a link between bioaccumulation and toxicity, but assumes that toxicity occurs when an organism accumulates metal concentrations in excess of its elimination and detoxification capacity (*40*). Subcellular compartmentalization, despite its technical limitations (*41*), combined with biodynamic modeling may be a means of discriminating among taxa based on susceptibility differences (*19*). For instance, net modeled Cd bioaccumulation varied 2.6 fold

among taxa. But when subcellular partitioning was considered, taxa varied approximately 5.3 fold in Cd concentrations predicted to occur in metal-sensitive compartments.

Relevance to Nature. Few field data are available to verify modeled Cd bioaccumulation for these taxa and its toxicological significance. Relative comparisons are possible for whole body Cd concentrations in *Claassenia, Hesperoperla*, and *Isogenoides* from the Clark Fork of the Columbia River (*42*). There, cadmium concentrations were similar among the three genera ($\sim$1.2 μg g^{-1} dry weight on average), although *Isogenoides* fell in the lower- to mid-range of the concentration distribution which would seem to agree with our relative ranking of $[Cd]_{ss}$. With respect to the distribution of these taxa along the metal contamination gradient, *Isogenoides* was common throughout the river, whereas *Claassenia* and *Hesperoperla* were generally found only in the less contaminated reaches. Cain et al. (2004) provided evidence that the distributions of several species in the Clark Fork (the species here were not considered in their study) were related to differences in their partitioning of metals (Cu, Cd, and Zn) into metal-sensitive compartments (*21*).

The use of aquatic insects in bioassessment should benefit greatly from the enhanced understanding of physiological differences among species. Here we show that species-specific physiological processes can be used to infer Cd susceptibility differences. While highlighting a major physiological difference between perlids and perlodids (dissolved uptake rates), we found substantial variability in this trait among perlids. Resh et al. (1975) emphasized the importance of species-level identification in the use of these insects in evaluating ecological conditions and commented on the limitations of designating whole taxonomic groups (typically family level or higher) as tolerant, or intolerant (*43*). The results of this study suggest that physiological variation within families may limit the efficacy of family level generalizations of tolerance. In general, a better understanding of physiological differences among aquatic insect species can only enhance the interpretive power of the bioassessments that utilize these important organisms. Finally, in light of the importance of dietary exposures in metal accumulation in many aquatic invertebrates, it seems appropriate to question whether water quality criteria generated from studies that ignore this important exposure route are adequately protective.

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Literature Cited

(1) Nriagu, J.; Pacyn, J. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* **1988**, *333*, 134−139.

(2) Rainbow, P. S. Trace metal concentrations in aquatic invertebrates: why and so what? *Environ. Pollut.* **2002**, *120*, 497−507.

(3) Clements, W. H.; Carlisle, D. M.; Lazorchak, J. M.; Johnson, P. C. Heavy metals structure benthic communities in Colorado mountain streams. *Ecol. Appl.* **2000**, *10* (2), 626−638.

(4) Wallace, J. B.; Webster, J. R. The role of macroinvertebrates in stream ecosystem function. *Annu. Rev. Entomol.* **1996**, *41*, 115−139.

(5) Hawkins, C. P.; Norris, R. H.; Hogue, J. N.; Feminella, J. W. Development and evaluation of predictive models for measuring the biological integrity of streams. *Ecol. Appl.* **2000**, *10* (5), 1456−1477.

(6) Barbour, M. T.; Gerritsen, J.; Snyder, B. D.; Stribling, J. B. *Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*, 2nd ed; U.S. Environmental Protection Agency, Washington, DC, 2006.

(7) Suter, G. W., II Applicability of indicator monitoring to ecological risk assessment. *Ecol. Indic.* **2001**, *1*, 101−112.

(8) Phillips, D. J. H.; Rainbow, P. S. The Biomonitoring of Trace Metals and Radionuclides. In *Biomonitoring of Trace Aquatic Contaminants*; Elsevier Applied Science: New York, 1992.

(9) Hare, Landis; Tessier, Andre; Croteau, Marie-Noele. A Biomonitor for tracking changes in the availability of lakewater cadmium over space and time. *Hum. Ecol. Risk Assess.* 2007.

(10) Luoma, S. N.; Rainbow, P. S. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environ. Sci. Technol.* **2005**, *39* (7), 1921−1931.

(11) Fisher, N. S.; Teyssie, J.-L.; Fowler, S. W.; Wang, W.-X. Accumulation and retention of metal in mussels from food and water: A comparison under field and laboratory conditions. *Environ. Sci. Technol.* **1996**, *30*, 3232−3242.

(12) Irving, E. C.; Baird, D. J.; Culp, J. M. Ecotoxicological responses of the mayfly *Baetis Tricaudatus* to dietary and waterbrone cadmium: Implications for toxicity testing. *Environ. Toxicol. Chem.* **2003**, *22* (5), 1058−1064.

(13) Wilding, J.; Maltby, L. Relative toxicological importance of aqueous and dietary metal exposure to a freshwater crustacean: Implications for risk assessment. *Environ. Toxicol. Chem.* **2006**, *25* (7), 1795−1801.

(14) Goulet, R. R.; Krack, S.; Doyle, P. J.; Hare, L.; Vigneault, B.; McGeer, J. C. Dynamic multipathway modeling of Cd bioaccumulation in Daphnia magna using waterborne and dietborne exposures. *Aquat. Toxicol.* **2006**.

(15) Munger, C.; Hare, L. Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus puntipennis*): Implications for predicting Cd bioaccumulation in nature. *Environ. Sci. Technol.* **1997**, *31*, 891−895.

(16) Roy, I.; Hare, L. Relative importance of water and food as cadmium sources to the predatory insect *Sialis velata* (Megalopter). *Can. J. Fish. Aquat. Sci.* **1999**, *56*, 1143−1149.

(17) Niyogi, S.; Wood, C. M. Biotic ligand model, a flexible tool for developing site-specific water qualtiy guidelines for metals. *Environ. Sci. Technol.* **2004**, *38* (23), 6177−6192.

(18) Brix, K. V.; DeForest David K.; Burger, M.; Adams, W. J. Assessing the relative sensitivity of aquatic organisms to divalent metals and their representation in toxicity datasets compared to natural aquatic communities. *Hum. Ecol. Risk Assess.* **2005**, *11*, 1139−1156.

(19) Buchwalter, D. B.; Cain, D. J.; Clements, W. H.; Luoma, S. N. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. *Environ. Sci. Technol.* **2007**, *41*, 1821−4828.

(20) Croteau, M.-N.; Luoma, S. N. Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand and biodynamics using enriched stable isotopes. *Environ. Sci. Technol.* **2007**, *41*, 3140−3145.

(21) Cain, D. J.; Luoma, S. N.; Wallace, W. G. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environ. Toxicol. Chem.* **2004**, *23* (6), 1463−1473.

(22) Wallace, W. G.; Lee, B.-G.; Luoma, S. N. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). *Mar. Ecol. Prog. Ser.* **2003**, *249*, 183−197.

(23) Wang, W.-X.; Rainbow, P. S. Subcellular partitioning and the prediction of cadmium toxicity to aquatic organisms. *Environ. Chem.* **2006**, *3*, 395−399.

(24) Mount, D. R.; Highland, T. L.; Mattson, V. R.; Dawson, T. D.; Lott, K. G. Use of the oligochaete, *Lumbriculus Variegatus*, as a prey organism for toxicant exposure of fish through the diet. *Environ. Toxicol. Chem.* **2006**, *25* (10), 2760−2767.

(25) Schlekat, C. E.; Lee, B.-G.; Luoma, S. N. Dietary Metals Exposure and Toxicity to Aquatic Organisms: Implications for Ecological Risk Assessment. In *Coastal and Estuarine Risk Assessment*; Newman, M. C., Roberts, M. H., Jr., Hale, R. C., Eds.; Lewis Publishers: Boca Raton, FL, 2002.

(26) Ritterhoff, J.; Zauke, G.-P. Potential role of metal-binding proteins in cadmium detoxification in *Themisto libellula* (Mandt) and *Themisto abyssorum* Boeck from the Greenland Sea. *Mar. Environ. Res.* **1998**, *45* (2), 179−191.

(27) Suzuki, K. T.; Sunaga, H.; Hatakeyama, S.; Sumi, Y.; Suzuki, T. Differential binding of cadmium and copper to the same protein

in a heavy metal tolerant species of mayfly (*Baetis thermicus*) larvae. *Comp. Biochem. Physiol.* **1989**, *94C* (1), 99−103.
(28) Cain, D. J.; Buchwalter, D. B.; Luoma, S. N. Influence of metal exposure history on the bioaccumulation and subcellular distribution of aqueous cadmium in the insect *Hydropsyche californica*. *Environ. Toxicol. Chem.* **2006**, *25* (4), 1042−1049.
(29) Wang, W.-X.; Fisher, N. S. Delineating metal accumulation pathways for marine invertebrates. *Sci. Total Environ.* **1999**, *237/238*, 459−472.
(30) Ng, T. Y. T.; Wang, W.-X. Modeling of cadmium bioaccumulation in two populations of the green mussel *Perna viridis*. *Environ. Toxicol. Chem.* **2005**, *24* (9), 2299−2305.
(31) *An Introduction to the Aquatic Insects of North America*, 3 ed.; Kendall/Hun Publishing Co.: Dubuque, IA, 1996.
(32) Evans, R. D.; Balch, G. C.; Evans, H. E.; Welbourn, P. M. Simultaneous measurement of uptake and elimination of cadmium by caddisfly (Trichoptera: Hydropsychidae) larvae using stable isotope tracers. *Environ. Toxicol. Chem.* **2002**, *21* (5), 1032−1039.
(33) Croteau, M.-N.; Hare, L.; Tessier, A. Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 1737−1746.
(34) Timmermans, K. R.; Spijkerman, E.; Tonkes, M.; Govers, H. Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 655−661.
(35) Seebaugh, D. R.; Estephan, A.; Wallace, W. G. Relationship between dietary cadmium absorption by grass shrimp (*Palaemonetes pugio*) and trophically available cadmium in amphipod (*Gammarus lawrencianus*) prey. *Bull. Environ. Contam Toxicol.* **2006**, *76* (1), 16−23.
(36) Cheung, M.; Wang, W.-X. Influence of subcellular metal compartmentalization in different prey on the transfer of metals to a predatory gastropod. *Mar. Ecol. Prog. Ser.* **2005**, *286*, 155−166.
(37) Wallace, W. G.; Luoma, S. N. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Mar. Ecol. Prog. Ser.* **2003**, *257*, 125−137.
(38) Wallace, W. G.; Lopez, G. R. Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. *Estuaries* **1996**, *19* (4), 923−930.
(39) Taylor, C. W. Calcium regulation in blowflies: Absence of a role for midgut. *Am. J. Physiol. Regul. Integr. Comp Physiol.* **1985**, *249*, 209−213.
(40) *Toxicity of Dietborne Metals to Aquatic Organisms*; SETAC Press: Lawrence, KS, 2006; p 1−303.
(41) Giguere, A.; Campbell, P. G.; Hare, L.; Couture, P. Sub-cellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (Perca flavescens) sampled along a polymetallic gradient. *Aquat. Toxicol.* **2006**, *77* (2), 178−189.
(42) Cain, D. J.; Luoma, S. N.; Carter, J. L.; Fend, S. V. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 2141−2154.
(43) Resh, V. H.; Unzicker, J. D. Water quality monitoring and aquatic organisms: The importance of species identification. *J. Water Pollut. Control Fed.* **1975**, *47* (1), 9−19.

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